PATENT COOPERATION TREATY

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From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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# PCT

## NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

| | |
|---|---|
| Date of mailing (day/month/year) | 30.10.2001 |
| Applicant's or agent's file reference | IMPORTANT NOTIFICATION |
| P 21059 | |

| International application No. | International filing date (day/month/year) | Priority date (day/month/year) |
|---|---|---|
| PCT/US00/19497 | 14/07/2000 | 13/07/1999 |

Applicant
AMYLIN PHARMACEUTICALS, INC. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

| Name and mailing address of the IPEA/ | Authorized officer |
|---|---|
| European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Danti, B<br>Tel.+49 89 2399-8161 |

Form PCT/IPEA/416 (July 1992)

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| P 21059 | | |
| International application No. | International filing date (day/month/year) | Priority date (day/month/year) |
| PCT/US00/19497 | 14/07/2000 | 13/07/1999 |

International Patent Classification (IPC) or national classification and IPC
G01N33/50

Applicant
AMYLIN PHARMACEUTICALS, INC. et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 12/02/2001 | 30.10.2001 |
| Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 | Authorized officer Giry, M Telephone No. +49 89 2399 7328 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/19497

## I. Basis of the report

1. With regard to the elements of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

**Description, pages:**

| | | |
|---|---|---|
| 1-29,31,32 | as originally filed | |
| 30 | with telefax of | 18/10/2001 |

**Claims, No.:**

| | | |
|---|---|---|
| 1-19 | with telefax of | 18/10/2001 |

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/19497

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 7, 9, 11, 13-19 |
| | No: | Claims | 1-6, 8, 10, 12 |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-19 |
| Industrial applicability (IA) | Yes: | Claims | 1-19 |
| | No: | Claims | |

2. Citations and explanations
see separate sheet

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US00/19497

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1 - Reference is made to the following documents :

D1: K. Miller et al. : 'Membrane-bound and solubilized brain $5HT_3$ receptors: improved radioligand binding assays using bovine area postrema or rat cortex and the radioligands $^3$H-GR65630, $^3$H-BRL43694, and $^3$H-LY278584.' Synapse, vol. 11, no. 1, 1992, pages 58-66

D2: B. A. Whelan et al. : 'Synthesis and structural, conformational, biochemical, and pharmacological study of new compounds derived from Tropane-3-spiro 4'(5')-imidazoline as potential 5-$HT_3$ receptor antagonists' J. Pharm. Sci., vol. 84, no. 1, January 1995, pages 101-108

D3: US-A-5 264 372, 23 November 1993, cited in the application

2 - **Novelty** - Art. 33(1) and (2) PCT :

2.1 Document D1 concerns the study of serotonine (5HT) receptors in bovine *area postrema* tissue. Document D1 discloses radioligands binding assays using bovine *area postrema* homogenates and the $5HT_3$ receptor antagonists $^3$H-GR65630 and $^3$H-BRL43694 in competition experiments (p. 59, col. 2, § "Radioligand binding studies of membrane-bound $5HT_3$ receptors") and presents representative competition curves for antagonists and agonists competed for specific $^3$H-GR65630 or $^3$H-BRL43694 binding (p. 61, Figures 5 and 6 ; p.62, Table II). Document D1 therefore appears to be novelty destroying for the subject-matter of claims 1-6, 8, 10 and 12.

2.2 Document D2 describes the synthesis of compounds 5(6)a-f derived from tropane-3-spiro-4'-imidazoline and the effects of said compounds on the binding of $^3$H-GR65630 to brain *area postrema* membranes in competition experiments (p. 103, col. 2, 3 last lines to p. 104, col. 2, line 18 ; Figures 4 and 5 ; p. 105, col. 2, lines 37-66). Thus, in light of document D2, the subject-matter of claims 1-6, 8, 10 and 12 cannot be regarded as novel.

**INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US00/19497

2.3 The available prior art documents disclose neither an assay method according to claims 7 and 9, a method for separating *area postrema* binding compounds wherein components of said *area postrema* are bound to a solid carrier (claim 12), nor a method of screening for a compound able to modulate a "biological function of the *area postrema* related to fuel homeostasis" (claims 14-20). Consequently, the subject-matter of claims 7, 9, 11 and 13-19 can be considered as new.

3 - **Inventive step** - Art. 33(1) and (3) PCT :

3.1 The two steps subject-matter of dependent claims 7 and 9 that further characterize the known methods of claims 1 and 2, respectively, fall within the customary practice followed by one skilled in the art. Thus, the subject-matter of claims 7 and 9 cannot be regarded as involving an inventive step.

3.2 Document D3 which is considered to represent the closest prior art document discloses methods for identifying or screening or characterizing or assaying or isolating known or candidates agonists and antagonists of amylin comprising binding assays utilizing preparations containing specific receptors for amylin. Membranes from the brain that contain high density receptors for amylin are used in the methods of the invention and as a source of amylin receptors (Abstract). The subject-matter of the present application differs from document D3 in that it concerns screening, identifying, characterizing, assaying and isolating candidate agonists and antagonists of different compounds. The problem to be solved by the present application can therefore be seen in providing screening, identifying, characterizing, assaying and isolating candidate agonists and antagonists of alternative compounds.

3.3 Document D3 discloses that the basal forebrain tissue is used as an amylin receptor preparation and may be bound to a solid phase and used in various affinity chromatography methods, for example for the purification of amylin or the evaluation of samples known or suspected to contain amylin, amylin agonists or amylin antagonists (col. 6, lines 52-57 ; col. 14, line 60 to col. 16, line 26). The selection of *area postrema* preparations as a source of receptors would be obvious to the skilled person since it appears to be well-known in the art that the

**INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET**



*area postrema* is a hindbrain region enriched in various receptors for peptides hormones (see p. 2, lines 22-28 of the description). The selection of this particular tissue does not appear to be linked to any unexpected effects. Therefore, the subject-matter of independent claim 11 cannot be considered as involving an inventive step.

3.4 Independent claim 13 concerns a method of screening for a "compound able to modulate a biological function of the *area postrema* related to fuel homeostasis" comprising adding a compound to said tissue preparation and measuring the effect of the compound on said biological function. Such methods comprising these two steps are customary in the field. Furthermore, it appears to be well-known in the art that receptors for hormones involved in this mechanism such as insulin, vasopressin, amylin, are located in this area of the brain (see p. 2, lines 22-28 of the description) and document D1 reports that $5HT_4$ receptor antagonists are effective antiemetic drug, especially useful in reversing the gastrointestinal disturbances (p. 58, col. 1, lines 8-11). Therefore, it appears that selecting *area postrema* to conduct the method in relation to a biological function related to fuel homeostasis would be obvious to one skilled in the art. Hence, the subject-matter of claim 13 cannot be considered as involving an inventive step.

3.5 In light of documents D1 and D3 teaching that amylin is a hormone isolated from pancreas and is associated with diabetes, claims 14-19 dependent on claim 13 do not appear to contain any additional technical feature which in combination with the features of the claim to which they refer can be regarded as involving an inventive step.

**Re Item VIII**

**Certain observations on the international application**

1. The application now comprises two claims numbered "claim 14" and no "claim 13". Therefore, the first claim 14 has been considered as claim 13.

**INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US00/19497

2. The Applicant's attention is drawn to the fact that features mentioned after "optionally" in claims 10 and 12 are regarded as optional features which have no limiting effects on the claims (Art. 6 PCT).

3. Since the method of claim 10 appears to be achieved by the same steps as those necessary to perform the method of claim 6, it appears that claim 10 is superfluous and should have been deleted (Art. 6 PCT).

the dorsal medulla oblongata (APX). Seven controls were similarly surgically treated, except the *area postrema* was left intact (SHAM). Following recovery from surgery, animals were anesthetized with halothane and subjected to a glucose-clamp procedure whereby plasma glucose was held constant by a glucose infusion varied in response to frequently determined plasma glucose concentration. After 60 minutes of glucose-clamp, 2mmol L-arginine was infused intravenously over 10 minutes. Plasma glucose, lactate, and insulin were measured for 90 min after L-arginine. There was a large increase in plasma insulin concentration in APX animals that was not observed in SHAM rats. These results demonstrate that pathways controlling insulin secretion, a key hormone involved in fuel homeostasis, include the *area postrema*.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. ~~Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention are defined by the scope of the claims~~.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in

PCT/US00/19497

Amylin Pharmaceuticals, Inc.

New claims:

1. An assay method for use in identifying or screening for compounds that stimulate or inhibit area postrema biological function, which comprises the steps of

(a) bringing together a test sample and an area postrema preparation, said test sample containing one or more test compounds;

(b) incubating said test sample and said area postrema preparation under conditions which would permit activation by said test compound of a biological process in, or the binding of said test compound to, said area postrema preparation; and

(c) identifying those test samples containing one or more test compounds which detectably activate, or bind to, said area postrema preparation.

2. The assay method of claim 1 which further comprises

(d) screening said test samples which detectably bind to said area postrema preparation for in vitro or in vivo stimulation or inhibition of area postrema mediated activity; and

(e) identifying those test samples which act as agonists or antagonists of said area postrema biological function.

3. The assay method of claim 1, wherein said area postrema preparation comprises isolated cells.

4. The assay method of claim 1, wherein said area postrema preparation comprises isolated membranes.

5. The assay method of claim 1, wherein said area postrema preparation comprises isolated tissue.

6. The assay method of claim 1, wherein said test samples which detectably bind to said area postrema preparation are identified by measuring the displacement of a labeled first ligand from said area postrema preparation by said test sample, and comparing the measured displacement of said first labeled ligand from said area postrema preparation by said test sample with the measured displacement of said first labeled ligand from said area postrema preparation by one or more known second ligands.

7. The assay method of claim 1, wherein said test sample contains more than one test compound, which further comprises the steps of

(d) preparing two or more additional test samples from said test sample, said additional test samples being characterized in that they contain a lesser number of test compounds than said test sample from which they were prepared; and

(e) repeating steps (a)-(d) as many times as required until the test compound or compounds which activate, or bind to, said area postrema preparation have been identified.

8. The assay method of claim 2, wherein said test samples which detectably bind to said area postrema preparation are identified by measuring the displacement of a labeled first ligand from said area postrema preparation by said test sample, and comparing the measured displacement of said first labeled ligand from said area postrema preparation by said test sample with the measured displacement of said first

labeled ligand from said area postrema preparation by one or more known second ligands.

9. The assay method of claim 8, wherein said test sample contains more than one test compound, which further comprises the steps of

(f) preparing two or more additional test samples from said test sample, said additional test samples being characterized in that they contain a lesser number of test compounds than said test sample from which they were prepared; and

(g) repeating steps (a)-(f) as many times as required until the test compound or compounds which bind to said area postrema preparation have been identified.

10. An assay method for determining the presence or amount of an area postrema binding compound in a test sample to be assayed for said compound, which comprises the steps of

(a) bringing together said test sample to be assayed and an area postrema preparation;

(b) measuring the ability of said test sample to compete against a labelled ligand for binding to said area postrema preparation; and, optionally,

(c) relating the amount of area postrema binding compound in said test sample with the amount of area postrema binding compound measured for a control sample in accordance with steps (a) and (b), said control sample being known to be free of any area postrema binding compound, and/or relating the amount of area postrema binding compound in said test sample with the amounts of area postrema binding compound measured for control samples containing known amounts of area postrema binding compound in accordance with steps (a) and (b), to

determine the presence or amount of area postrema binding compound in said test sample.

11. A method for separating area postrema binding compounds from a sample, which comprises the steps of

(a) bringing together said sample and an area postrema preparation, said area postrema preparation comprising components of said area postrema bound to a solid carrier; and

(b) separating any area postrema binding compound which is bound to said area postrema preparation from the remainder of said test sample which is unbound.

12. A method for screening a biological substance for the presence of components of said area postrema, which comprises the steps of

(a) bringing together said biological substance with first area postrema binding compound;

(b) bringing together said biological substance with a second area postrema binding compound;

(c) optionally bringing together said biological substance with one or more additional area postrema binding compounds; and

(d) determining the relative binding affinities of said area postrema binding compounds for said area postrema preparation in said biological substance.

13. A method of screening for a compound able to modulate a biological function of the area postrema related to fuel homeostasis, comprising adding a compound to an area postrema preparation, and measuring the effect on said biological function.

14. The method of claim 13, wherein said area postrema preparation comprises one or more materials selected from the group consisting of area postrema, nucleus tractus solitarius material, and material from the dorsal motor nucleus of the vagus nerve.

15. The method of any of claims 13 or 14, wherein said material is selected from the group consisting of a membrane, a cell and a tissue.

16. The method of claim 13, wherein said biological function is modulation of pancreatic endocrine secretion.

17. The method of claim 13, wherein said biological function is modulation of body energy content.

18. The method of claim 13, wherein said biological function is linked to a metabolic disease.

19. The method of claim 18, wherein said metabolic disease is selected from the group consisting of diabetes and obesity.